

served. Sometimes little but the imprint of the fossil remains, and even this is usually much distorted, particularly in the head region. This being the case, the identifications must be based on the general proportions and on the relationships of superficial parts to a greater degree than on osteological details. Dr. Jordan's extremely wide knowledge of modern fishes must, therefore, have been almost indispensable in the work he has done. Despite this advantage, however, the relationships of a considerable number of the forms described could not be definitely determined. Occasionally, the estimate of kinship was widely altered in subsequent papers. For example, a fish first regarded as a herring was (from better material) later referred to the *Syngnathidae*, while a supposed labrid was later shown to represent a clupeid. It must be noted, however, that in the later papers a greater attention to the skeletal features which are yet apparent—particularly the interneurals and interhaemals, as related to one another and to the fin rays—has added an increased definiteness to the identification and the determination of relationships.

The reviewer feels constrained to express his opinion that throughout the work (as in so many treatises on fossil fishes) too many imperfect and incomplete impressions were considered. To name and to attempt the classification of fossil remains of clearly indeterminate relationship can hardly serve to advance our knowledge of extinct faunas. In the case under review, a close study of the best preserved remains of these Tertiary fishes (some show exquisite detail) would in his opinion have yielded the same generalizations, and would have added stability to the nomenclature, and prevented the intrusion into the work of certain elements of unnecessary doubt.

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LABORATORY APPARATUS AND METHODS

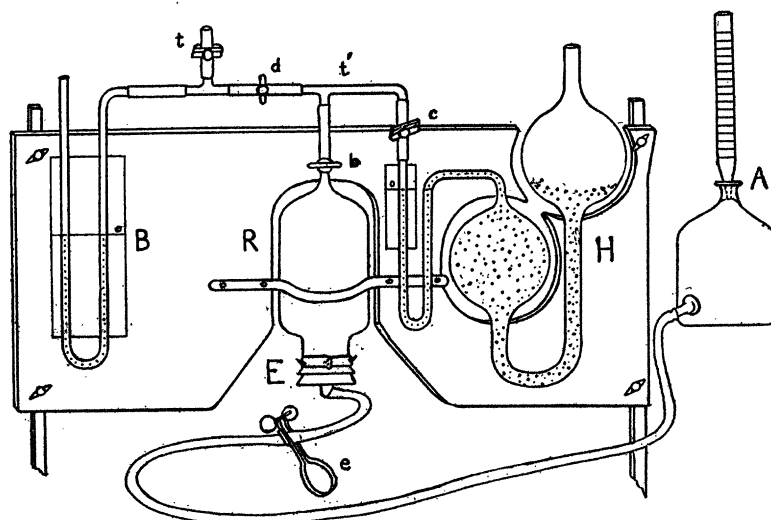
RESPIRATORY EXCHANGE OF THE FROG

THREE years ago the writer began using a very simple class demonstration to show the change in volume imposed upon respired air during the respiratory exchange of the frog in its winter or early spring condition. A short metal tube was soldered over a hole made in the lid of a one-pint Mason fruit jar. A frog was placed in the jar and the jar-lid was screwed down air tight. About three minutes were allowed for the changing volume of air in the jar, occasioned by the slightly warmer body of the frog, to reach equilibrium, and then, by a suitable rubber tube, the metal tube of the jar-lid was connected with

a water manometer. The frog was allowed to breathe the air in the jar for one hour to one and one half hours, during which time the water in the manometer arms gradually changed level several centimeters in such direction as to show a decrease in volume of the air in the jar. The frog appeared to experience no special discomfort in breathing the confined air for that length of time.

This year the thought occurred that this experiment might be easily modified and extended so as to afford a very good method (for class use) of determining the respiratory quotient of the frog. The apparatus now used is represented in the accompanying figure. It consists of the following glass parts: a water manometer, *B*; two heavy T-tubes of small bore, *t* and *t'*; a special respiration chamber, *R*, of 250 cc capacity; an elbow-tube through a rubber stopper, *E*; a special Hempel gas absorption pipette, *H*, of 250 cc capacity, and an ordinary aspirating bottle, *A*, of 250 cc capacity having a Mohr burette (25 cc graduated to 1/10 cc) inserted through a rubber stopper in its top in such manner that, as water rises from the aspirating bottle into the burette, air can not be trapped beneath the stopper. These parts are connected by rubber tubing as illustrated. The neck of the respiration chamber at *E* has an opening of 45 mm in diameter and takes a No. 10 rubber stopper. A metal collar around this neck of the chamber bears two hooks over which a pliable wire may be tightly looped to hold the stopper in exact place during the experiment. The chamber may be closed above with a glass stop cock, *b*. At *c*, *d* and *t* are screw pinch cocks, and at *e* is a Mohr's pinch cock. A solution of one part of KOH to two parts of water is used in the Hempel pipette. The aspirating bottle with its burette and its tubing is filled with pure water. It was found that acidulated water (HCl) may not be used. Even when the acid used is weak enough to seem not especially irritating to the skin of the frog, it nevertheless appears to be absorbed by the skin so that CO₂ is liberated from the alkaline carbonates of the blood. Thus the volume of the gas in the respiration chamber slowly but constantly increases until the frog dies.

When an experiment is to be started, the apparatus (as described) and the frog to be used are brought into a room where the temperature is comparatively constant and kept until they are as nearly as possible the same temperature as the air of the room. Then cock, *c*, is closed and cocks *b*, *d* and *t* are opened. The frog is introduced into *R* and the stopper *E*, is fastened securely in place with care not to place the hand on *R* so as to change its temperature. Cock, *e*, is opened to pass just enough water into *R* to cover the stopper, *E*, and seal it. Under these conditions, the manometer will read level



Apparatus used for showing the change in volume of air respired by a frog, and for determining the R. Q.

at atmospheric pressure. Cock, *t*, is now closed. For two or three minutes, thereafter, it will usually be observed that the outer limb of the manometer rises, indicating that the volume of the air with the frog is increasing a little. This appears to be due to the frog's body being very slightly warmer than the air. The volume ceases to increase within about two minutes, as a rule; then *t* is momentarily opened and closed again to restore the manometer to zero at atmospheric pressure, and the experiment begins. Gradually the manometer will show that the volume of the air with the frog is slowly decreasing. The best results are obtained, with a respiration chamber of this size, by terminating the experiment in 20 to 25 minutes, depending somewhat on the activity of the frog. It seems best in other words not to allow the difference reading of the manometer arms to exceed eight or nine centimeters. The reason for this will be pointed out in a moment. When the respiration period is to be terminated, the burette of *A* is read, then cock, *e*, is opened to allow the passage of just enough water into *R* to restore the zero level of the manometer. The burette of *A* is again read. Cock, *d*, is now closed, and *c* is opened. The bottle, *A*, is raised and cock, *e*, is opened. Water flows into *R* until most of the respired air is driven into the KOH pipette. The air is then returned to *R*, by lowering *A*, for a moment. This operation is repeated three times—all the air being sent into *H* the third time—care being used that no water is driven beyond *b* and that no KOH is drawn beyond *c*. When the water goes over the frog's head, he promptly stops lung breathing, and suffers no ill effects, until the air is returned from the Hempel pipette. As the air is returned the last time, the KOH solution is carefully stopped just as it rises to

the zero point of the Hempel absorption pipette. Cock, *c*, is then closed and cock, *d*, is opened. The aspirating bottle, *A*, and cock, *e*, are now manipulated, if necessary, until the manometer stands at zero. The burette of *A* is read again. The manipulation for CO_2 absorption should occupy four or five minutes. Even then the manometer level will be apt to change at first in such direction as to show a slight increase in the volume of the air with the frog, but this should not make a difference of more than 0.2 cc in the final reading of the burette at *A*. During the course of the experiment, the percentage of CO_2 in the air confined in the respiration chamber gradually increases until slightly abnormal amounts of CO_2 begin to be retained in the blood of the frog. This CO_2 is given off by the frog comparatively rapidly after the CO_2 -free air from the Hempel pipette is returned to the respiration chamber, hence the necessity of returning the air three times. However, if the respiration period has been continued for an hour or so (too long, for a chamber of this size), the abnormal accumulation of CO_2 in the frog will be so great as to require considerable time for it to be given up—thus too greatly prolonging the time used in absorbing CO_2 .

Let *n* equal the first reading of burette, *A*, with the manometer level at atmospheric pressure.

Let *m* equal the second reading of burette, *A*, at the end of the respiration period, after the manometer is restored to the zero level.

Then the difference (*m* — *n*) equals the volume of the oxygen taken up by the frog, less the volume of the carbon dioxide given off in respiration.

Let *p* equal the reading of burette, *A*, after CO_2 absorption in the Hempel pipette.

Then *p* — *m* equals the volume of CO_2 given off in respiration.

$p - m + (m - n)$ equals $p - n$ equals the volume of oxygen retained by the frog during the respiration period.

$$R. Q. \text{ equals } \frac{CO_2}{O_2} \text{ equals } \frac{p - m}{p - n}$$

A series of nine experiments carried out in the manner described on the same frog gave the following values for the respiratory quotient: 0.71; 0.72; 0.76; 0.80; 0.65; 0.69; 0.72; 0.70; 0.78. Of course the method has its obvious limitations as to accuracy and must not be urged, in that respect, in comparison with those methods which permit the animal to breathe fresh air throughout the experiment and also allow the respiration period to be terminated on an exact moment. Nevertheless, the results obtained are fairly uniform and the method does afford some advantages as a laboratory experiment for illustrating the facts involved in the respiratory exchange. The manometer visibly impresses the student with the fact that in case of an animal oxidizing protein and fat the volume of the oxygen used in respiration is greater than the volume of the carbon dioxide given off. The experiment may be carried out in a comparatively short time and the operation is simple—only one absorbent fluid being necessary. A "cold blooded" animal is made use of, so that the temperature of the respired air is so slightly above that of the surrounding air as not to interfere with the immediate volumetric work, as may be the case when a mammal is used.

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LABELING MICROSCOPE SLIDES

THE method developed by the writer of preparing labels for microscope slides has been used sufficiently by the departments of plant pathology and botany at the University of Wisconsin to warrant the hope that it will prove of value to others.

In the preparation of a large number of, or even a few, microscope slides for class use satisfactory labeling of the finished slides is often an important problem. Printed labels are expensive, while printing or writing even a few duplicate labels is both time-consuming and irksome, and requires unusual skill for satisfactory results.

The method herein described produces in any quantity neat, accurate labels in the form of photographic prints the exact size of a standard slide label and gummed ready for use. In addition the process is simple, rapid and inexpensive, and requires very little special equipment.

The original copy from which the negative is made may be drawn to any scale, preferably two or three

times the desired size. The standard slide label is fifteen sixteenths of an inch square. A piece of white bristol board or drawing paper is ruled with a 3H pencil into squares of three times the linear dimensions of the slide label. As a matter of economy of time and materials 12 or 24 different labels are prepared at one time on a single large sheet of paper. Twelve labels arranged in three rows of four each can be photographed on a 4 x 5 plate, leaving about one half inch margin all around for convenience in handling. Similarly, 24 labels in four rows of six each will go on a 5 x 7 plate.

The data are now lettered in the squares in pencil, using penciled guide lines which may be ruled for the entire sheet at one time. After having been checked for errors, the labels are inked with black waterproof ink, using a ruling pen for straight lines and Barch-Paysant lettering pens (Keuffel and Esser Co.) for the lettering. Pens No. 4 and No. 5 are suitable for lettering a label enlarged three times (Fig. 1, A), while No. 5 and No. 6 are suitable for a label enlarged two times (Fig. 1, C). The necessity of allowing for reduction in the width of lines is emphasized by Fig. 1 in which B and D are photographic reductions of A and C. It also shows that slight imperfections in the lettering are minimized by reduction.

The completed sheet of labels is cleaned with art gum, photographed on a process plate, and developed in a contrast developer (hydroquinone). To avoid distortion of the image on the plate the center of the copy should be approximately on the optical axis of the camera lens, and perpendicular to it. The image is easily tested for size by measuring with a slide labeled on the ground glass, or for squareness, by measuring the diagonals, which should be equal in length. As many prints as are desired can now be quickly made from the negative, which may be preserved for future needs. A dull or semi-gloss printing paper is preferred by the writer to a glossy paper. Solar, a thin, matt-surfaced printing paper made by the Defender Photo Company, gives labels of practically the same thickness and surface as the commercial labels.

The adhesive is applied to the back of the prints before the labels are cut apart. An adhesive prepared from animal glue was found superior to the various commercial liquid glues tested. The following formula is a slight modification of one suggested by Mr. Wilbur Jones, of the Forest Products Laboratory, Madison, Wisconsin. A good grade of animal glue, which comes in small flakes, is dissolved in twice its weight of water in a water bath. When the glue is thoroughly dissolved, ten per cent. of glycerine (by volume) is added to make it flexible when dry, and one half per cent. of betanaphthol as a